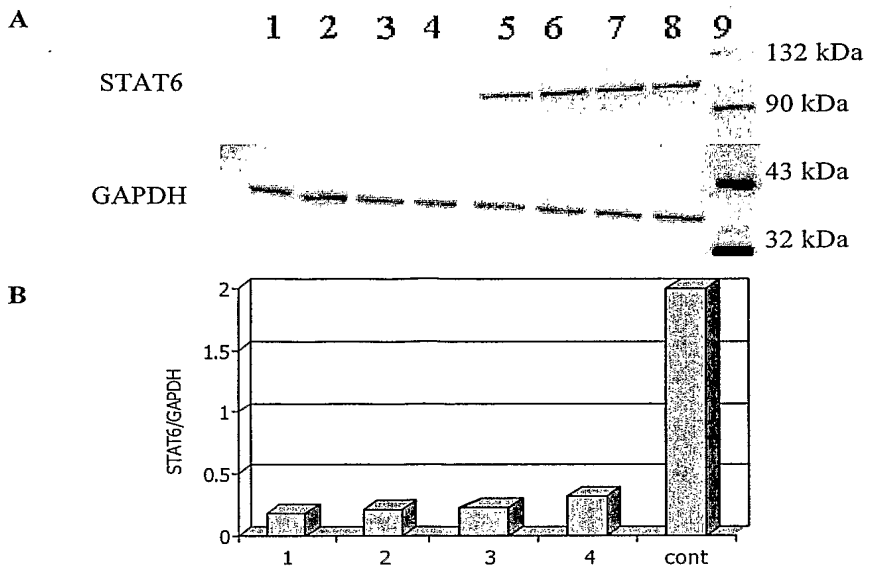
DNA sequence encoding mRNA	siRNA Duplex Structure
STAT6(1): AAGCAGGAAGAACTCAAGTTT →	5'-GCAGGAAGAACUCAAGUUUtt-3' 3'-ttCGUCCUUCUUGAGUUCAAA-5'
[SEQ ID No.15]	[SEQ ID No.1]
STAT6(2): AAACAGTACGTTACTAGCCTT →	5'-ACAGUACGUUACUAGCCUUtt-3' 3'-ttUGUCAUGCAAUGAUCGGAA-5'
[SEQ ID No.16]	[SEQ ID No.2]
STAT6(3): AAGAATCAGTCAACGTGTTGT →	5'-GAAUCAGUCAACGUGUUGUtt-3' 3'-ttCUUAGUCAGUUGCACAACA-5'
[SEQ ID No.17]	[SEQ ID No.3]
STAT6(4): AAAGCACTGGAGAAATCATCA →	5'-AGCACUGGAGAAAUCAUCAtt-3' 3'-ttUCGUGACCUCUUUAGUAGU-5'
[SEQ ID No.18]	[SEQ ID No.4]

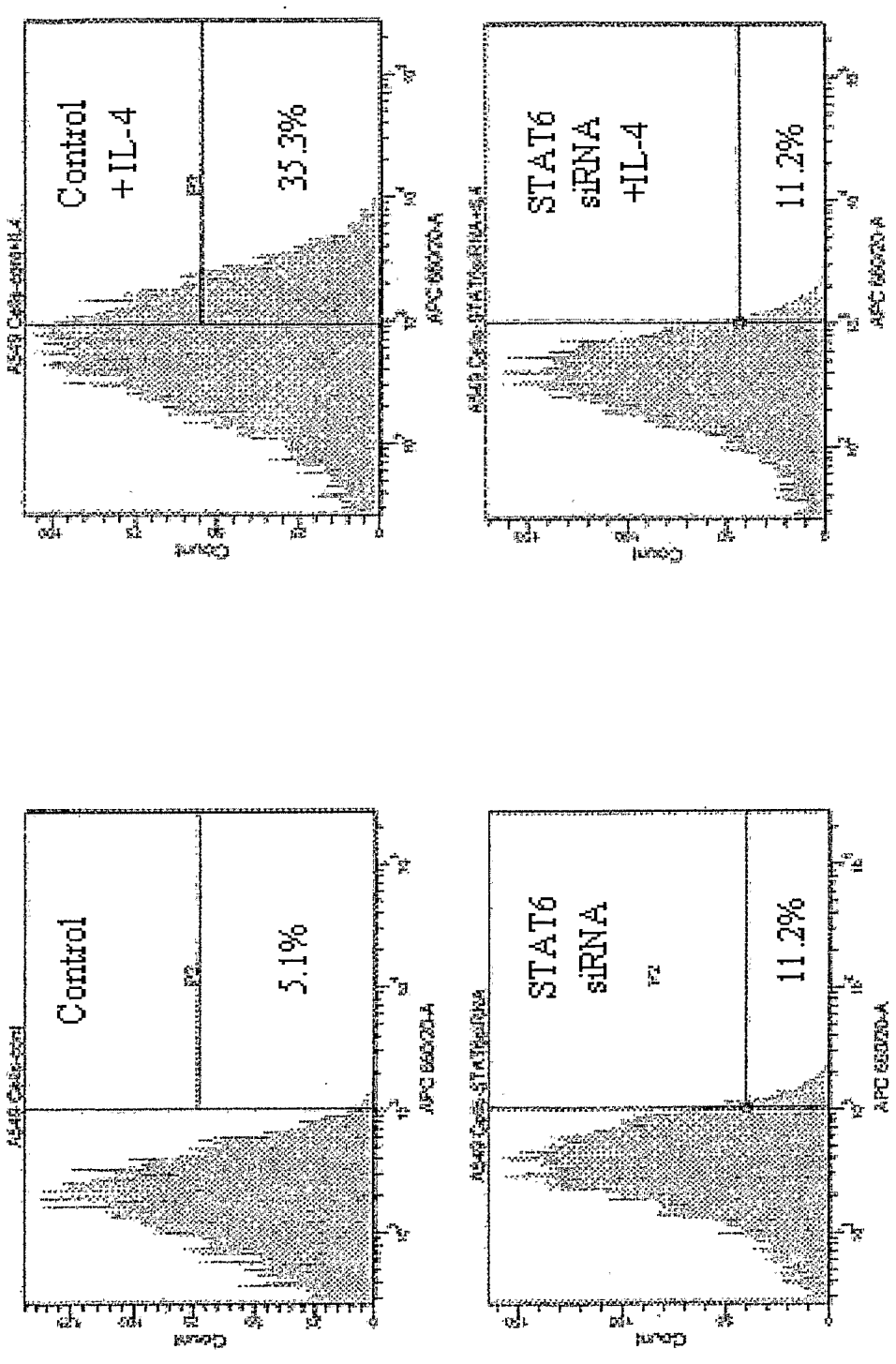
Figure 1



1= STAT6(1) siRNA
2= STAT6(2) siRNA
3= STAT6(3) siRNA
4= STAT6(4) siRNA
5= GAPDH siRNA
6= scGAPDH siRNA
7= untreated cells
8= untreated + IL-4
9= molecular weight markers

1= STAT6(1) siRNA
2= STAT6(2) siRNA
3= STAT6(3) siRNA
4= STAT6(4) siRNA
cont = scGAPDH siRNA

Figure 2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5.1%
Control
+IL-4
35.3%
STAT6
siRNA
11.2%
STAT6
siRNA
+IL-4
11.2%

Figure 3

NM 003153 [gi:23397677]

LOCUS NM_003153 3993 bp mRNA linear PRI 27-OCT-2004
DEFINITION Homo sapiens signal transducer and activator of transcription 6, interleukin-4 induced (STAT6), mRNA.
ACCESSION NM_003153
VERSION NM_003153.3 GI:23397677
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from BC005823.2 and BQ028928.1.
On Oct 1, 2002 this sequence version replaced gi:21536302.

Summary: The protein encoded by this gene is a member of the STAT family of transcription factors. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein plays a central role in exerting IL4 mediated biological responses. It is found to induce the expression of BCL2L1/BCL-X(L), which is responsible for the anti-apoptotic activity of IL4. Knockout studies in mice suggested the roles of this gene in differentiation of T helper 2 (Th2) cells, expression of cell surface markers, and class switch of immunoglobulins.

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CDS 272..2815
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/note="STAT, interleukin4-induced; transcription factor IL-4 STAT;
go_component: nucleus [goid 0005634] [evidence IEA];
go_function: signal transducer activity [goid 0004871] [evidence IEA];
go_function: transcription factor activity [goid 0003700]

Figure 4

[evidence TAS] [pmid 10747856];
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go_process: regulation of transcription from Pol II
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Figure 4 (continued)

polyA_site 3978
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Figure 4 (continued)

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2881 cttgctcatg ccctgccaag cagcagatgg ggagggtgcc ctcctatccc cacctactcc
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Figure 4 (continued)

NM 009284 [gi:6678154]

LOCUS NM_009284 3213 bp mRNA linear ROD 28-OCT-2004
DEFINITION Mus musculus signal transducer and activator of transcription 6 (Stat6), mRNA.
ACCESSION NM_009284
VERSION NM_009284.1 GI:6678154
KEYWORDS .
SOURCE Mus musculus (house mouse)
ORGANISM Mus musculus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. The reference sequence was derived from L47650.1.
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go_function: DNA binding [goid 0003677] [evidence IEA];
go_function: signal transducer activity [goid 0004871] [evidence IEA];
go_function: transcription factor activity [goid 0003700] [evidence IEA];
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Figure 5

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Figure 5 (continued)

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3181 ctctgggagg gagaaaaagg aaaaagtggg tat [SEQ ID No.12]

Figure 5 (continued)

XM 343223 [gi:34865760]

LOCUS XM_343223 2442 bp mRNA linear ROD 24-OCT-2003
DEFINITION Rattus norvegicus similar to signal transducer and activator of transcription 6 (LOC362896), mRNA.
ACCESSION XM_343223
VERSION XM_343223.1 GI:34865760
KEYWORDS .
SOURCE Rattus norvegicus (Norway rat)
ORGANISM Rattus norvegicus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.
COMMENT MODEL REFSEQ: This record is predicted by automated computational analysis. This record is derived from an annotated genomic sequence (NW_047777) using gene prediction method: GNOMON, supported by EST evidence.
Also see:
Documentation of NCBI's Annotation Process

FEATURES Location/Qualifiers
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SDAFCYNMASALLSATVQRLQASAGEQGKGSSLLQHISTLESIYQRDPLKLVATIRQI

LQGEKKAVIEEFHHLPGPFHRKQEELKFTTALGRLQHRVRETRILRESLQQGTKTAQV

SLKNLIDPPANGTGPSEDLATMLQGTVGDLEATQALVLKRIQIWKRQQQLAGNGTPFE

Figure 6

ESLAGLQERCESLVEIYSQLQQEIGAASGELEPKTRASLISRLDEVLRTLVTSSFLVE

KQPPQVLKTQTKFQAGVRFLLGLQFLGTSAKPPLVRADMVTEKQARELSLPQGSGAGV

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MLGPNKHLIQLQALSLPLVVIVHGNQDNNAKATILWDNAFSEMDRVPFVVAERVPWEK

MCETLNLKFMAEVGTSRGLLPEHFLFLAQKIFNDNSLSIEAFQHRCVSWSQFNKEILL

GRGFTFWQWFDGVLDLTKRCLRSYWSDRLIIGFISKQYVTSLLLNEPDGTFLLRFSDS

EIGGITIAHVIRGQDGSSQIENIQPFSAKDLSIRSLGDRIRDLAQLKNLYPKKPKDEA

FRSHYKPEQMGKDGRGYVSTTIKMTVERDQPLPTPEPQMPAMVAPYDLGMAPDASMQL

SSDMVPHLQMPPTMSQISMPFDQPHPQGLLQCQSQEHAVSSPEPLLCSDVTMAEDSCL

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GISMSHLDLRTNPSW" [SEQ ID No.13]

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/gene="LOC362896"
/note="STAT_prot; Region: STAT protein, protein interaction domain. STAT proteins (Signal Transducers and Activators of Transcription) are a family of transcription factors that are specifically activated to regulate gene transcription when cells encounter cytokines and growth factors. STAT proteins also include an SH2 domain pfam00017"
/db_xref="CDD:pfam02865"

misc_feature 370..813
/gene="LOC362896"
/note="STAT; Region: STAT protein, all-alpha domain. STAT proteins (Signal Transducers and Activators of Transcription) are a family of transcription factors that are specifically activated to regulate gene transcription when cells encounter cytokines and growth factors. STAT proteins also include an SH2 domain pfam00017"
/db_xref="CDD:pfam01017"

misc_feature 817..1581
/gene="LOC362896"
/note="STAT_bind; Region: STAT protein, DNA binding domain. STAT proteins (Signal Transducers and Activators of Transcription) are a family of transcription factors that are specifically activated to regulate gene transcription when cells encounter cytokines and growth factors. This family represents the DNA binding domain of STAT, which has an ig-like fold. STAT proteins also

Figure 6 (continued)

include an SH2 domain pfam00017"
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/gene="LOC362896"
/note="SH2; Region: SH2 domain"
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ORIGIN
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61 gactttccac aacacctgcg gcatcttctg gccgaatggc tggagaatca gccctgggag
121 ttcctggttg gttcagatgc tttctgttac aacatggcta gtgccctact ttctgccact
181 gtccagcgtc ttcaggcctc tgcaggagag caggggaaag gaagcagcct cttgcagcac
241 atcagcacct tggagagcat ctatcagagg gacccccctga agctggtggc caccatcaga
301 caaatacttc aaggggagaa aaaagctgtt atagaagagt tccaccacct gccagggccc
361 ttccatcgaa agcaggaaga actcaagttt actacagccc tgggcaggct tcagcaccga
421 gtaagggaga ccaggattct ccgagaatct ctgcagcagg ggaccaagac tgcgcaagtg
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541 acgatgctgc aggggactgt gggggacttg gaggccaccc aggctctagt gctgaaaagg
601 attcagattt ggaagcggca acagcagctg gcagggaatg gcacaccctt tgaggagagc
661 ctggcagggc tgcaggagag gtgtgaaagc ctggtggaaa tttattccca gctgcagcag
721 gagattggag cagccagtgg ggagcttgag cccaagaccc gggcatcgct cataagccgt
781 ctggatgaag tcctgcgaac cctcgtgacc agctctttcc tggtggagaa gcagccccca
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961 gccagagaac taagcctgcc ccaggggtct ggggctggag tggagagcac aggagagatc
1021 atgaacaata ctgtacctct ggagaacagt gttcctggga actgctgctc tgccctcttc
1081 aagaacctgc tcctgaagaa aatcaagcgc tgtgagcgga agggtacaga gtctgtcacc
1141 gaagagaagt gcgctgtgct cttctctacg agcttcatgc tgggccccaa caaacacctc
1201 atccagcttc aggccctgtc tctgcccttg gtggtcatcg ttcatggcaa ccaagacaac
1261 aatgccaaag ctaccatcct gtgggataat gccttctctg agatggaccg agtgcccttt
1321 gtggtagctg agcgagtgcc ctgggagaaa atgtgtgaaa ctctgaacct caagtttatg
1381 gctgaggtgg ggaccagccg gggactgcta ccagaacact tcctgttcct ggcccagaag
1441 atcttcaatg acaacagcct tagcatagag gcctttcagc accgctgtgt gtcttggtca
1501 cagttcaaca aggagattct actgggccga ggcttcactt tttggcagtg gtttgatggt
1561 gtcctggacc tcactaaacg ctgtcttcgg agctactggt cagatcggct gatcatcggc
1621 tttatcagta agcaatatgt cactagcctt ctcctcaacg agccagatgg aaccttcctc
1681 ctccgcttta gcgactctga gattgggggc atcaccattg cccatgtcat ccggggtcag
1741 gatggctcct cacagataga gaacatccag ccgttttctg ccaaagacct atccattcgc
1801 tcactggggg accgaatccg agatcttgct caattaaaaa acctctaccc caagaaaccc
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1981 ccccagatgc ctgccatggt ggccccttat gatcttggaa tggcccctga tgcttccatg
2041 caactcagct cagatatggt gcctcacctt caaatgcccc ccaccatgag ccagataagc
2101 atgccctttg accagcctca tccccagggc ctgctccagt gccagtccca ggagcatgcg
2161 gtgtccagcc ctgaaccctt gctgtgttca gatgtcacta tggcggaaga cagctgccta
2221 actcagcctg tgcaaggttt cccccagggc acctgggtca gcgaaggcat gtaccctccc
2281 ctgatgcctc ccactgaaca ggacctcacc aagcttctcc tagagggcca aggggaaggt

Figure 6 (continued)

2341 ggaggatcca tagggactca gcccctcctg caaccatctt cttatgggca atcggggatc
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Figure 6 (continued)

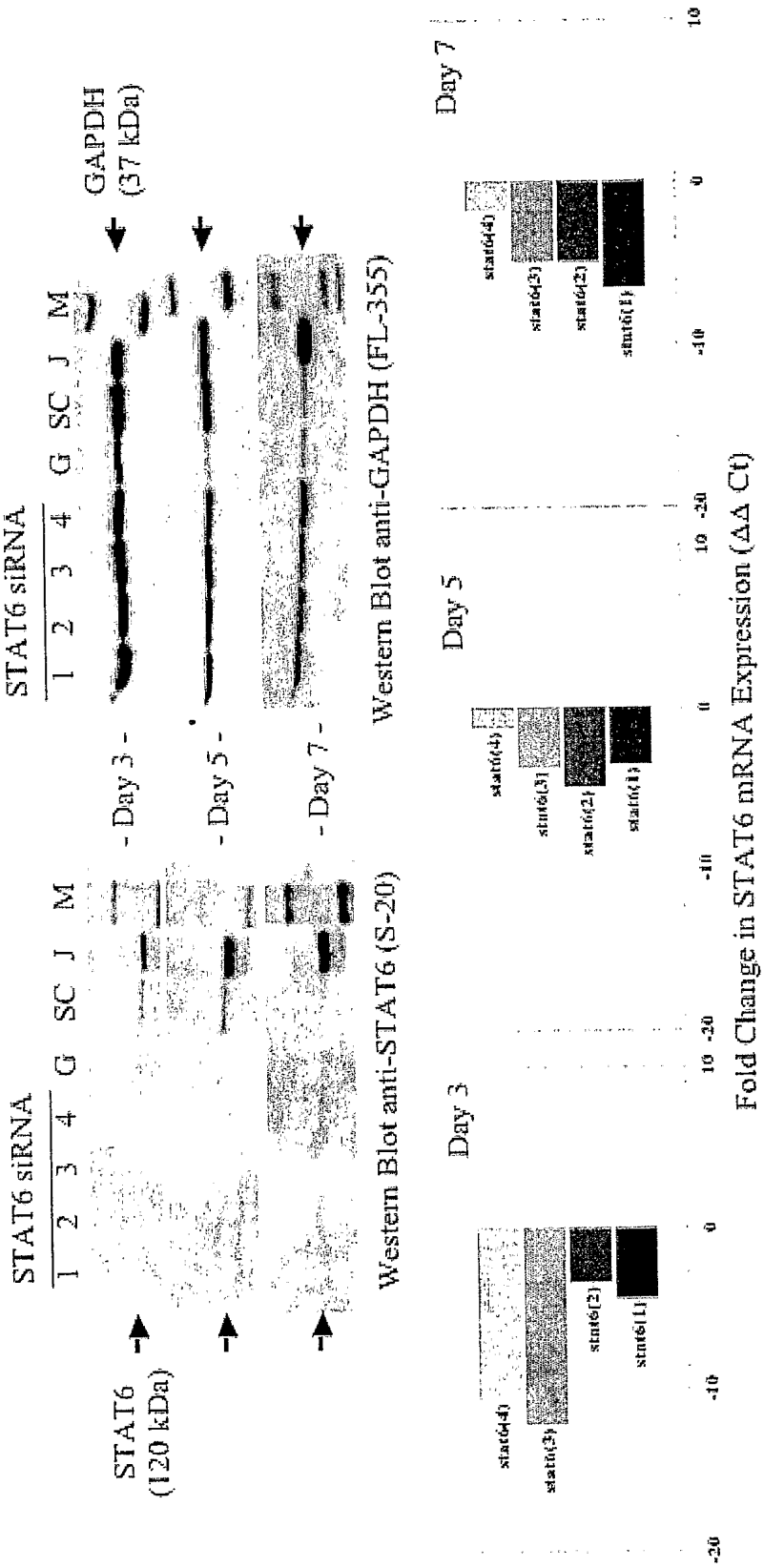
STAT6 siRNA
1 2 3 4 G SC J M
STAT6 (120 kDa)
- Day 3 -
- Day 5 -
- Day 7 -
Western Blot anti-STAT6 (S-20)
GAPDH (37 kDa)
Western Blot anti-GAPDH (FL-355)
Day 3
Day 5
Day 7
Fold Change in STAT6 mRNA Expression (ΔΔ Ct)

Figure 7

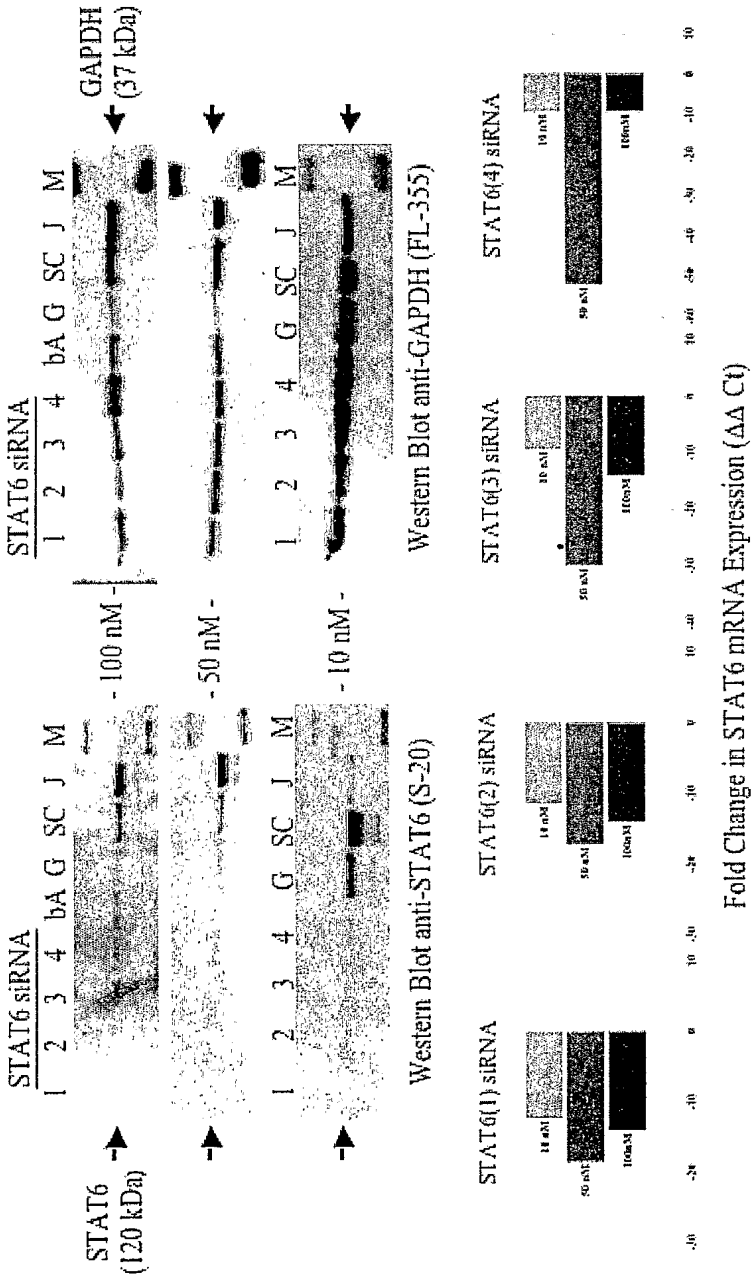
STAT6 siRNA
1 2 3 4 bA G SC J M
- 100 nM -
- 50 nM -
- 10 nM -
STAT6 (120 kDa)
GAPDH (37 kDa)
Western Blot anti-STAT6 (S-20)
Western Blot anti-GAPDH (FL-355)
STAT6(1) siRNA
STAT6(2) siRNA
STAT6(3) siRNA
STAT6(4) siRNA
Fold Change in STAT6 mRNA Expression (ΔΔ Ct)

Figure 8

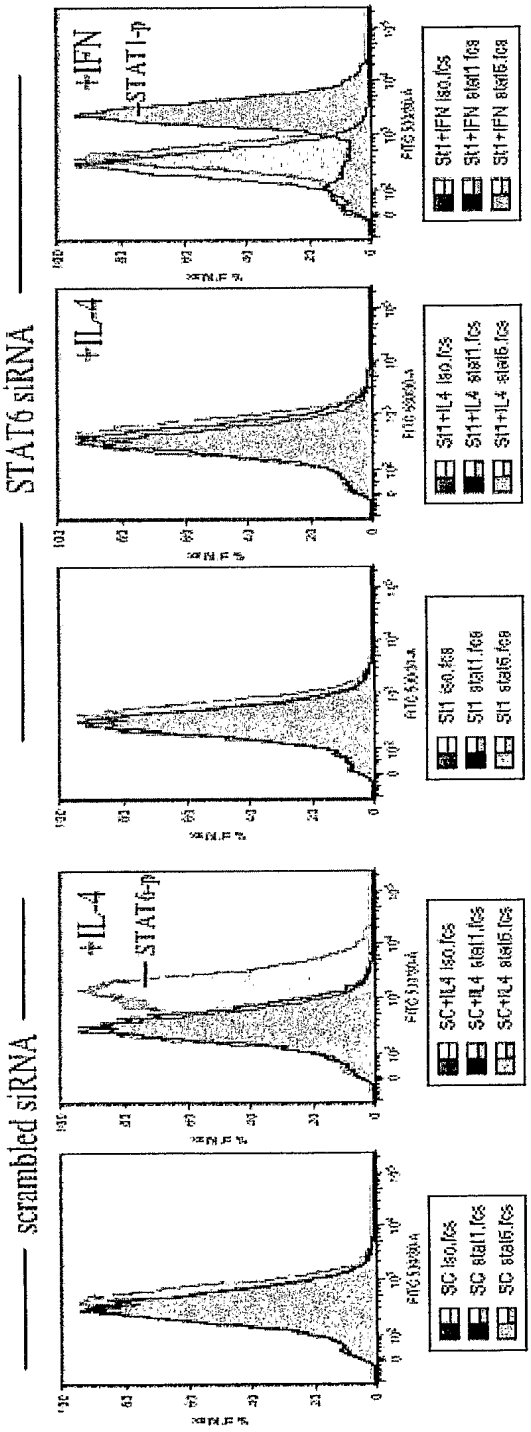
scrambled siRNA
STAT6 siRNA
+IL-4
STAT6-p
+IL-4
+IFN
STAT1-p
SC iso.fcs
SC stat1.fcs
SC stat6.fcs
SC+IL4 iso.fcs
SC+IL4 stat1.fcs
SC+IL4 stat6.fcs
St1 iso.fcs
St1 stat1.fcs
St1 stat6.fcs
St1+IL4 iso.fcs
St1+IL4 stat1.fcs
St1+IL4 stat6.fcs
St1+IFN iso.fcs
St1+IFN stat1.fcs
St1+IFN stat6.fcs

Figure 9

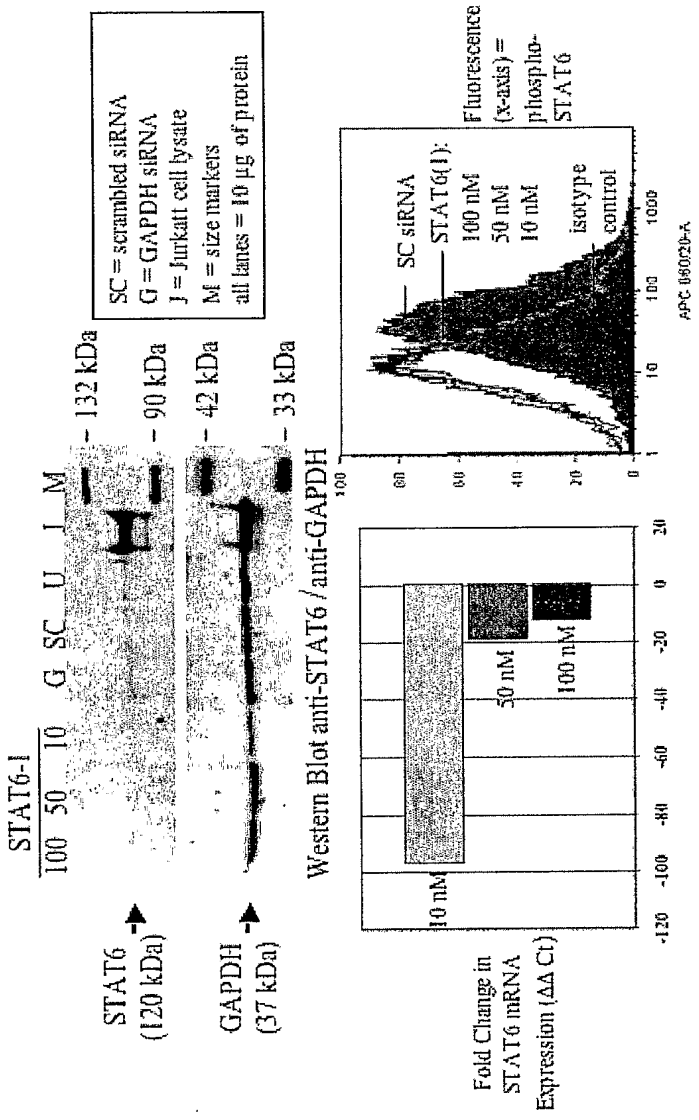
STAT6-I
100 50 10 G SC U J M
132 kDa
90 kDa
42 kDa
33 kDa
STAT6 (120 kDa)
GAPDH (37 kDa)
SC = scrambled siRNA
G = GAPDH siRNA
J = Jurkatt cell lysate
M = size markers
all lanes = 10 µg of protein
Western Blot anti-STAT6 / anti-GAPDH
SC siRNA
STAT6(I):
100 nM
50 nM
10 nM
isotype control
Fluorescence (x-axis) = phospho-STAT6
10 nM
50 nM
100 nM
Fold Change in STAT6 mRNA Expression (ΔΔ Ct)

Figure 10

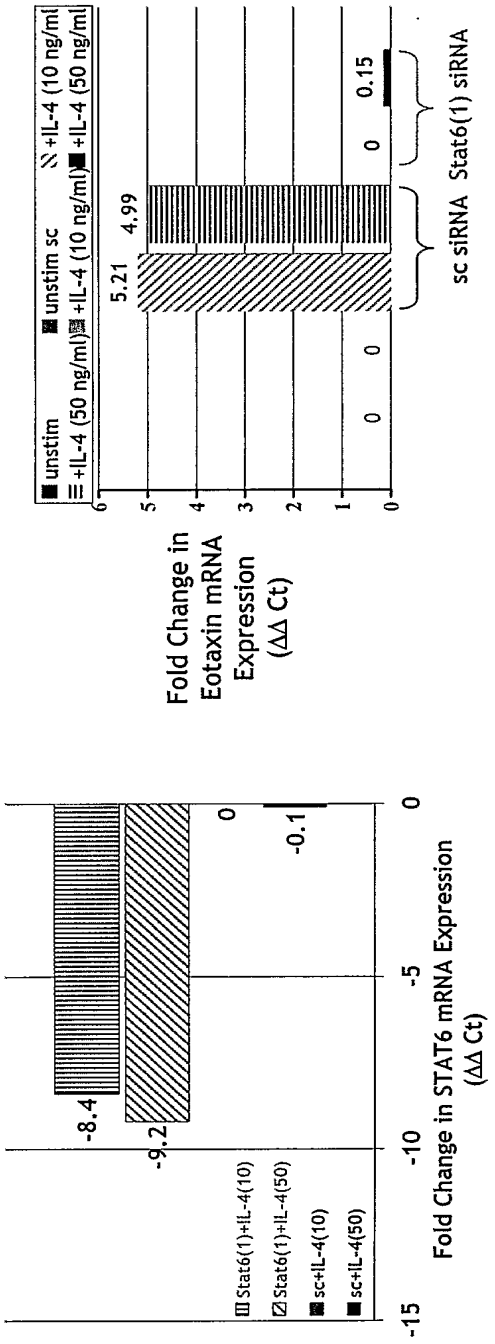
unstim
unstim sc
+IL-4 (10 ng/ml)
+IL-4 (50 ng/ml)
+IL-4 (10 ng/ml)
+IL-4 (50 ng/ml)
Fold Change in Eotaxin mRNA Expression (ΔΔ Ct)
5.21
4.99
0
0
0
0.15
sc siRNA
Stat6(1) siRNA
-8.4
-9.2
0
-0.1
Stat6(1)+IL-4(10)
Stat6(1)+IL-4(50)
sc+IL-4(10)
sc+IL-4(50)
Fold Change in STAT6 mRNA Expression (ΔΔ Ct)

Figure 11